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REVIEW

# Mechanisms and mediators of hypertension induced by erythropoietin and related molecules

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## ABSTRACT

Hypertension is a common but frequently overlooked adverse effect of erythropoietin (EPO) therapy. Underreporting of hypertension with EPO is likely due to either more aggressively managing hypertension through the prescription of antihypertensive drugs or closer attention to dry weight. The purpose and focus of this review is to critically evaluate the mechanisms of EPO-induced hypertension. Preclinical data are considered first, followed by clinical data where available. Mediated by a variety of molecules, there is an imbalance in the vascular tone favoring net vasoconstriction that mediates EPO-induced hypertension. Animal studies show the primary importance of chronic kidney disease in the genesis of EPO-induced hypertension. Preclinical studies show deranged regulation of the nitric oxide, endothelins and porstanoids and the sympathoadrenal and renin–angiotensin pathways as causes of EPO-induced hypertension. Human studies suggest that EPO administration is also associated with increased responsiveness to catecholamines and angiotensin II on vascular tissue; in addition, hypoxia-induced vasodilation may be impaired in those with EPO-induced hypertension. There is little evidence for EPO as a direct vasoconstrictor or its effect on blood viscosity as a mechanism of EPO-induced hypertension. EPO-induced hypertension, at least in part, appears to be independent of an increase in hemoglobin, because experiments show that hemoglobin may be increased by EPO without an increase in blood pressure (BP) by simply treating the animals with EPO-binding protein and that treatment with EPO in the setting of iron deficiency may not increase hemoglobin but may still increase BP. However, experimental data are not consistent across studies and better mechanistic designs are needed, especially in patients with chronic kidney disease, to dissect the precise mechanism of EPO-induced hypertension. Animal studies suggest that hypoxia-inducible factor stablizers may induce hypertension by provoking calcification and augmenting chronic intermittent hypoxia as occurs in sleep apnea. Others show that there may be an antihypertensive effect via kidney repair. Whether these drugs will alter the risk of hypertension compared with EPO remains to be seen.

**Keywords:** anemia, erythropoietin, HIF stablizers, hypertension, nitric oxide

Hypertension is a common but frequently overlooked adverse effect of erythropoietin (EPO) therapy [1–6]. Underreporting of hypertension with EPO is likely due to either more aggressively managing hypertension through the prescription of antihypertensive drugs, closer attention to dry weight [7, 8] or lack of ambulatory blood pressure (BP) measurements [9]. The purpose and focus of this review is to critically evaluate the mechanisms of EPO-induced hypertension. Preclinical data are considered first, followed by clinical data where available.

## IMPORTANCE OF CHRONIC KIDNEY DISEASE (CKD) IN EPO-INDUCED HYPERTENSION

The first study to show that CKD was an important determinant for EPO-induced hypertension was reported from France in 1995 [10]. Male Wistar rats underwent subtotal nephrectomy at 6 weeks of age, were allowed to recover for 6 weeks and were then treated for 3 weeks with either vehicle or EPO. Time controls were sham-operated animals that did not undergo nephrectomy and were treated with either vehicle or EPO. As expected, subtotal nephrectomy in animals increased plasma creatinine and systolic BP and induced a decrease in hematocrit. Treatment with EPO provoked a significant increase in hematocrit regardless of nephrectomy. EPO treatment increased systolic BP significantly in the nephrectomized group by 37.5 ± 11 mmHg. There was no significant increase in systolic BP in the nonnephrectomized EPO-treated or vehicle-treated rats. Furthermore, no relationship was seen between the change in hematocrit and the change in systolic BP [10]. Using a very similar design as above, a 1997 study from Quebec confirmed the

 

central importance of reduced renal mass in the development of hypertension [11]. EPO had no effect on BP in the control rats despite an increase in hematocrit in rats without nephrectomy, yet had a large effect in those with a nephrectomy.

The subtotal nephrectomy model provides a consistent observation for both studies in that by itself—over the time course studied—it did not provoke hypertension. Furthermore, there was no increase in BP in the EPO-treated sham-operated animals. It required both subtotal nephrectomy and EPO to provoke hypertension. Whether nephrectomy activated the renin–angiotensin system, caused volume excess or caused endothelial dysfunction to induce hypertension was not examined in these studies, but these possibilities were subsequently explored in other models and will be discussed further below.

Human observations are consistent with animal data. Although EPO is abused by some athletes to enhance performance, hypertension or hypertensive crises are not reported. Similarly, the incidence of hypertension in zidovudine-treated human immunodeficiency virus–positive anemic patients or among those undergoing cancer chemotherapy is low. However, EPO-induced hypertension is very common among those with CKD, including those on dialysis [6].

## HEMOPOIETIC VERSUS HYPERTENSIONOGENIC EFFECTS OF EPO

Whether BP tracks with hemoglobin or not remains controversial. In a 1988 report, among anemic rats with reduced renal mass, preventing anemia of renal failure with EPO aggravated systemic hypertension [12]. In contrast, a low-iron diet that maintained hemoglobin at a lower level prevented the development of hypertension, hyperfiltration, glomerulosclerosis and proteinuria [12]. In more recent animal experiments, hypertension did not track with an increase in hemoglobin [13]. If EPO is administered to anemic animals with chronic renal failure, but hemoglobin is kept stable by feeding an iron-deficient diet, hypertension still occurs. In blood vessels harvested from these animals treated with EPO, vasodilatory responses to nitric oxide (NO) donors were impaired but response to several vasoconstrictors was normal. Among patients on long-term hemodialysis, treatment with iron to increase hemoglobin was not associated with parallel increases in BP [14].

Human data are similarly inconclusive. Some studies show that although an increase in hemoglobin is dose dependent, an increase in BP is not [1, 15]. On the other hand, during the first 5 weeks of administration of EPO the change in hemoglobin concentration was directly related to an increase in diastolic BP ($r = 0.42$, $P < 0.001$) [3].

To understand the hypertensionogenic effects of EPO, consideration of the biology of the EPO receptor expressed in the nonhemopoietic tissues is needed.

## EPO RECEPTOR AND ITS RELEVANCE TO EPO-INDUCED HYPERTENSION

Mutation of EPO receptor is lethal *in utero* [16]. However, EPO receptor null mice can survive simply by expressing the EPO receptor in the hemopoietic tissue only [16]. Despite the lack of the EPO receptor in the endothelium, heart and brain, these mice, somewhat surprisingly, develop normally. Using genetic engineering to rescue EPO receptor null mice, two groups of mice were created with varying amounts of EPO receptor expressed in the hemopoietic tissue [16]. In one group, the expression of EPO receptor was 40% of normal and in the second group was 120% of normal. As expected, the circulating EPO level was higher when the EPO receptor was lower. However, contrary to expectations, in response to induced anemia the time to peak plasma EPO concentrations was delayed in both groups of mice. This suggests that the extra-hemopoietic EPO receptor may play an important role in the regulation of plasma EPO concentration.

There is substantial evidence that blood vessels express the EPO receptor. In a 1994 report, mRNA for the EPO receptor was identified in human umbilical vein endothelial cells [17]. These endothelial cells lacked erythroid precursors because they lacked the α-globin and γ-globin chains associated with hemoglobin. Furthermore, antibodies to the extracellular portion of the EPO receptor stained the vascular endothelium of the placenta and the umbilical cord. Cultured endothelial cells proliferate in response to EPO [18, 19]. The ability to proliferate is seen at concentrations of EPO as low as 1 U/mL [19], which is often achieved with intravenous administration of the drug [20]. The ability of human umbilical vein endothelial cells to migrate is also induced by EPO [19].

A series of studies performed by investigators in Japan among mice expressing EPO receptor in only the hemopoietic tissue demonstrate the importance of EPO receptor expression outside hemopoietic tissue [16] such as heart, lungs and the limbs [21]. Experiments in these mice lacking the EPO receptor outside hemopoietic tissue display several cardiac, pulmonary and vascular anomalies as follows: (i) Following coronary ligation, compared with wild-type mice, there was greater infarct size, due in part to accelerated cardiomyocyte apoptosis [22]. (ii) When pressure overload was induced by aortic banding, there was greater cardiac dilatation and impairment in cardiac contractility, due in part to reduced vascular endothelial growth factor expression and reduced myocardial capillary density [23]. In response to hypoxia, there was an increased risk of the development of pulmonary hypertension due to reduced mobilization of the endothelium progenitor cells [24]. (iv) In response to hind-limb ischemia, there was abrogation of the expression of vascular endothelial growth factor and its receptor, impairment in the recruitment of endothelial progenitor cells and bone marrow–derived proangiogenic cells and thereby reduced neovascularization [25].

Some data suggest that the effects on hemopoiesis and vasoconstriction may be mediated by different epitopes on the EPO molecule [26]. Rodents treated with EPO, when also treated with an EPO binding protein, had an increase in hemoglobin but their BP did not change. Furthermore, treatment of the above animals with the EPO binding protein and an antibody to this protein increased hemoglobin but did not change BP. These data suggest that the BP increasing effect of EPO and the hemopoietic effect of the EPO molecule may be mediated by different parts of the same molecule.

EPO can also be engineered by targeting it more specifically to the hemopoietic EPO receptor and reducing its ability to

bind to nonhemopoietic tissues [27]. A synthetic EPO molecule has been created that interacts minimally with the EPO receptor [27]. To selectively target EPO to the hemopoietic tissue, it was tethered to an antibody that specifically binds glycophrin A, which is highly expressed on red blood cells [27]. Compared with wild-type mice, when this new molecule was given to mice carrying the human glycophorin A gene, the half-life of engineered EPO was prolonged, reticulocyte response was augmented and platelet effects were minimized [27]. The authors speculate that EPO receptor expression on maturing megakaryocytes may create an off-target prothrombotic state. In their study, since the total platelet count and reticulated platelets were both reduced in comparison to darbepoietin, the new molecule may reduce the risk of thrombotic side effects. The promise of an engineered EPO with less BP raising effect has yet to be realized.

## MOLECULAR MECHANISMS OF EPO-INDUCED HYPERTENSION

The mechanisms of EPO-induced hypertension are incompletely understood. Figure 1 summarizes putative mediators and their effects on BP are discussed further.

### Evidence against direct effect of EPO on vascular smooth muscle or endothelium

Among untreated patients with essential hypertension, serum EPO concentration correlates with both systemic vascular resistance and 24-h ambulatory BP [28]. This has led to speculation that EPO may have direct vasoconstrictive effects. In order to interpret the results of cell culture studies, it is important to compare the plasma concentrations of EPO achieved after a bolus dose in humans with that used in preclinical studies. A study describing the pharmacokinetics of EPO in hemodialysis patients notes that the peak concentration of EPO when given as an intravenous bolus injection was 0.768 U/mL with 50 U/kg EPO and 2.434 U/mL with 150 U/kg EPO [20].

In 1991, a group of investigators from Muenster, Germany demonstrated that resistance arterioles from the kidneys and intestine could be made to vasoconstrict when exposed to EPO in doses ranging from 10 to 200 U/mL [29]. In 1993 the same group demonstrated that intracellular calcium is increased in cultured vascular smooth muscle cells in a dose-dependent manner upon incubation with EPO [30]. The vascular smooth muscle cells were incubated with 100 U/mL or 250 U/mL EPO. It should be noted that these concentrations of EPO are ∼100–200-fold higher than the peak concentrations achieved with intravenous EPO administration [20]. The physiological and clinical relevance of these observations is therefore unclear.

In humans, EPO by itself does not appear to have any effect on vasoconstriction. In a double-blind cross-over study in nine hemodialysis patients, Hon *et al.* [31] administered either EPO or saline intravenously. BP was measured every 5 min for 60 min following treatment. Between treatments, no differences

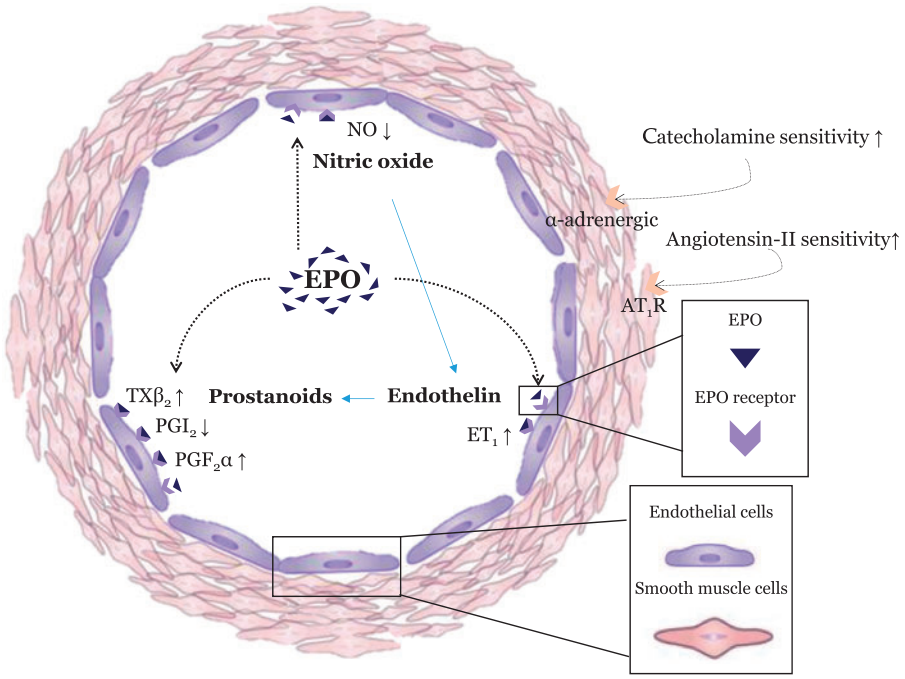


**FIGURE 1:** Putative mechanisms of EPO-induced hypertension through its receptors on the endothelial cells, especially in the setting of CKD, can trigger endothelial dysfunction and vasoconstriction. The NO pathway is severely deranged in response to EPO; ADMA concentrations increase and cGMP release with NO donors is impaired. Inhibitors of NOS severely elevate BP and provoke mortality in rodents with excess EPO expression. NO can also trigger ET-1 release, which itself is a potent vasoconstrictor. Prostanoids are downstream to endothelin and there is a net imbalance favoring vasoconstriction. The effects on the renin–angiotensin system are complex, but in preliminary studies vasoconstriction to angiotensin II is enhanced when patients are given EPO. Similarly, compared with patients not on EPO, vasoconstriction to catecholamines is enhanced when patients are treated with EPO. Additional pathways such as volume excess and other mechanisms discussed in the text are not depicted in the figure.

were seen [31]. A 2004 study in 41 dialysis patients reported an increase in mean arterial pressure from 103 to 105 mmHg after a single injection [32]. Although the change was only 2 mmHg, it was reported as significant; without a blinded control group, the likelihood of false discovery is high [32]. A 2013 study used subcutaneous arteries isolated from a gluteal biopsy from 17 patients with Stage 4 CKD [33]. The response to endothelial-dependent vasodilatation was tested *ex vivo* by evaluating acetylcholine-induced vasodilatation. These blood vessels were exposed to varying EPO concentrations at 0, 1, 10 and 20 IU/mL [33]. The difference in endothelial function was minimal when comparing 0 and 1 IU/mL but stark at higher concentrations. However, such higher concentrations are supra-pharmacologic and it is unclear if they have relevance to EPO biology. Furthermore, the effects of EPO may be mediated via endothelin, because using an endothelin type A receptor antagonist abrogated the effects of EPO [33].

### Endothelin-1

Rats with reduced renal mass have increased endothelin-1 (ET-1) expression in the aorta, mesenteric artery and renal cortex [34, 35]. These rats have increased 24-h urinary excretion of ET-1 [36]. EPO induces release of ET-1 from endothelial cells in culture [18, 37]. In contrast to the cell culture experiments, EPO administered to 5/6 nephrectomized rats does not increase the circulating concentration of ET-1 despite an increase in BP [38, 39]. This may be because the expression of ET-1 provoked by EPO is organ specific. EPO-treated rats with reduced renal mass have no increase in ET-1 in the mesenteric artery or renal cortex, yet the aortic content of ET-1 is increased [39, 40]. The increase in aortic ET-1 was further augmented by the NO synthase (NOS) inhibitor L-arginine analog $N^G$-nitro-L-arginine methyl ester (L-NAME). This suggests that NO has an important effect on abrogating the expression of ET-1 in large vessels and is an upstream process [40]. Abrogation of oxidative stress in EPO-treated nephrectomized rats by tempol also reduces tissue levels of ET-1, hypertension and renal injury [41]. This suggests that oxidative stress is also an upstream process to ET-1-generation in the kidney. However, as discussed below, prostanoids may be a downstream mediator of ET-1 induced vasoconstriction. Mice specifically expressing ET-1 in the endothelium, when treated with EPO even without nephrectomy, have an increase in BP, impairment of endothelial function, resistance artery remodeling and aortic inflammation and oxidative stress [42]. These adverse effects can be blocked with exercise over 8 weeks.

Consideration of the tissue-specific expression of endothelin receptors is needed to appreciate the endothelin biology. Rats with reduced renal mass have reduced expression of the vasodilatory endothelin receptor type B ($ET_B$) in the aorta, mesenteric artery and renal cortex [34, 35]. Furthermore, in rats with reduced renal mass, EPO increases the vascular and renal cortex expression of $ET_B$ receptor mRNA [43]. This parallels the increase in $ET_B$ receptor density in the endothelium of rats [43]. Compared with wild-type mice, mice heterozygous for the $ET_B$ receptor have a greater increase in BP with EPO [43]; mice heterozygous for the $ET_A$ receptor have BP responses that are similar to wild-type mice. In rats with subtotal nephrectomy and EPO-induced hypertension, treatment with a selective $ET_A$ receptor antagonist is more effective than placebo in abrogating the increase in BP [44]. In contrast, the nonselective $ET_A/ET_B$ receptor antagonist, bosentan had no effect in preventing EPO-induced hypertension.

In patients with CKD treated chronically with EPO, ET-1 concentration is increased [45–48]. In a cross-sectional study of 44 end-stage renal disease patients [24 hemodialysis, 20 continuous ambulatory peritoneal dialysis (CAPD)], of which half were on EPO therapy, plasma ET-1 concentration directly correlated with systolic BP [49]. In EPO-treated patients on CAPD, plasma ET-1 concentrations correleated with mean BP [50]. This correlation is not seen in those not treated with EPO [50]. However, the acute administration of EPO does not increase ET-1 concentration [31]. Vasoconstriction induced by infusion of ET-1 is similar in patients with CKD before and after treatment with EPO, thus the vasoconstriction dose–response relationship to ET-1 is not altered with EPO-treatment [51].

In summary, treatment of anemia of CKD with EPO uncovers the endothelial dysfunction through increased production of vascular ET-1. Reduced expression of the vasodilatory $ET_B$ receptor due to genetic heterogeneity or cross talk by other endothelium-derived vasoactive autacoids such as NO, prostanoids and angiotensin II may play an important role in the genesis of EPO-induced hypertension [35].

### Prostanoids

Prostanoids play an important role in maintaining vascular tone and renal sodium handling. Thromboxane $A_2$ ($TXA_2$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have vasoconstrictor activity, whereas $PGI_2$ is vasodilatory. $TXB_2$ is a stable metabolite of $TXA_2$, whereas 6-keto-$PGF_{1\alpha}$ is a stable metabolite of $PGI_2$ [37]. In a 2005 study, rats with reduced renal mass who became anemic and hypertensive as expected also had an increase in vascular and renal concentration of $TXA_2$ and $PGI_2$ [52]. In these animals, treatment with EPO further aggravated hypertension and stimulated vascular and renal $TXA_2$ and $PGI_2$ [52]. Pretreatment with aspirin blocks both the synthesis of $TXA_2$ and $PGI_2$ and therefore had no net effect on EPO-induced hypertension [52].

In contrast to systemic exposure of EPO, rabbit aortic rings incubated with EPO show an increase in $TXA_2$ but a reduction in $PGI_2$ [18, 37]. In human renal artery *in vitro* as well as rabbit aortic rings, incubation with EPO leads to an increase in the synthesis of constrictor prostanoids such as $TXB_2$ and $PGF_{2\alpha}$ but a reduction in the vasodilatory prostaglandin $PGI_2$ [53]. However, the concentration of EPO used to incubate these rings was far beyond the physiological range. $TXB_2$ was increased and 6-keto-$PGF_{1\alpha}$ unchanged in the aorta of rats with reduced renal mass treated with EPO [54]. An antagonist of thromboxane, ridogrel, abrogated hypertension in EPO-treated animals but did not alter the plasma concentration of ET-1 [54]. An antagonist of ET-1, ABT-627, was even more effective than ridogrel in treating hypertension; moreover, it also reduced the concentration of $TXB_2$ in the aorta [54]. Thus prostanoids may serve as a downstream mediator of vasoconstriction in response to ET-1 activation. The imbalance in vasoconstrictor to vasodilatatory prostanoids may lead to a net increase in vascular

resistance and therefore hypertension. The use of antiplatelet therapy has been postulated to prevent the development of hypertension among patients treated with EPO [55]; the mechanism of the antihypertensive effect remains unclear.

### NO

NO is a potent endothelium-derived vasodilator and plays an important role in the genesis of hypertension in CKD. NO has important effects on the endothelium, blood vessels, kidneys and BP. Each of the effects are discussed below.

**Endothelium.** In uremia, NO synthesis in the endothelium is blunted. Suprapharmacologic EPO exposure to endothelial cells in culture media results in a reduction in endothelial NOS [56] and high concentrations (such as 10–200 U EPO/mL) provoke an increase in the NOS inhibitor asymmetric dimethylarginine (ADMA) in a dose-dependent manner; this is accompanied by reduced activity of dimethylarginine dimethylaminohydrolase (DDAH) [57]. DDAH is an enzyme that breaks down ADMA, generating citrulline and dimethylamine, and is the major mechanism of ADMA removal [58]. *In vivo* studies reveal that compared with saline-treated controls, Balb/c mice with intact kidneys injected with more reasonable exposure to EPO (such as 30 U every other day for 10 weeks) show ADMA concentrations that were 46% higher [59]. In comparison, symmetric dimethyl arginine (SDMA) concentrations are unchanged. Of note, there is no mechanism of direct synthesis of ADMA; free ADMA is generated by proteolysis of methylated proteins [58]. Free ADMA can inhibit endothelial cell NOS and cause endothelial dysfunction and has been linked to poor outcomes in CKD.

**Blood vessels.** In rabbit, aortic rings harvested from animals with intact kidneys, acetylcholine-induced vasodilatation, which is dependent upon an intact endothelium, was not blunted when rings were incubated with EPO for 30 min [53]. In sharp contrast, among rats with reduced renal mass, the release of cyclic guanosine monophosphate (cGMP) by nitrate donors of aortic rings is augmented. However, this augmentation is blunted when rats were treated with EPO [60]. The endothelial and inducible NOS protein mass is reduced in these rats regardless of EPO treatment, but when treated with felodipine, the protein mass in the aorta is restored. Thus the presence or absence of renal failure has an important effect on the role of EPO in blood vessels; calcium-channel blockade may have at least partially restored the abnormalities.

**Kidney effects.** Rats with intact renal mass upon treatment with EPO display an increase in renal NO; this is noted by an increase in urinary cGMP [61] and urinary nitrates [62]. Rats with reduced renal mass have a reduction in the urinary nitrate excretion rate that is further reduced with EPO treatment [63]. The endothelial and inducible NOS protein mass is reduced in rats with subtotal nephrectomy regardless of EPO treatment, but when treated with felodipine the protein mass is restored [63]. When mice with intact renal mass are given EPO, although ADMA concentrations in plasma are increased, the urinary excretion of NO metabolites is also increased [59]. Similar findings are seen in rats with intact kidneys treated with EPO [64]. In fact, mice treated with EPO for 10 weeks have an increased kidney expression of NOS1 and NOS2 despite the EPO-induced increase in ADMA [59]. Similarly, rats with intact kidneys made hypertensive with EPO have increased urinary excretion of nitrites and nitrates [65]. The renal vasodilatory response to acetylcholine (endothelium dependent) and sodium nitroprusside (endothelium independent) are both similar to vehicle-treated rats [65]. Thus, reduced renal mass is necessary to uncover the effects of EPO.

**BP effects.** Inhibitors of NOS can exacerbate hypertension in EPO-treated animals even when the renal mass is not reduced [61]. Among animals treated with EPO, exposure to L-NAME, an inhibitor of NOS, results in hypertension [40], the magnitude of which is greater when renal mass is reduced [40]. In rats in whom CKD is induced by ligating renal arteries, the restoration of endothelial NOS by gene delivery improves NO release and prevents the development of hypertension [66]. In rats with reduced renal mass and EPO-induced hypertension, the treatment of hypertension with felodipine partially restores the abnormalities in NO metabolism [63].

The critical importance of NO in EPO-induced hypertension has been studied in a transgenic mouse model [67]. Transgenic mice overexpressing human EPO were generated. Despite hematocrit levels of 80%, adult transgenic mice did not develop hypertension or thromboembolism because of a compensatory increase in endothelial NOS levels, NO-mediated endothelium-dependent relaxation and circulating and vascular tissue NO levels. Administration of L-NAME led to vasoconstriction, an increase in vascular resistance, hypertension and death of transgenic mice; the wild-type siblings developed hypertension but did not show increased mortality.

A translational research study elucidated the mechanism of endothelial dysfunction in 56 patients on hemodialysis treated with EPO [68]. Endothelial progenitor cells, which the investigators state reflect endothelial cell function, were isolated from these patients and mRNA levels for both the full EPO receptor and a spliced, truncated EPO receptor were measured. The authors note that activation of the full EPO receptor triggers a signaling cascade that ultimately terminates in cGMP and NO production and subsequent vasodilation. However, it was observed that in patients with EPO-induced hypertension there was a positive correlation with the spliced, truncated variant of the receptor. This led to the conclusion that the truncated variant of the receptor serves as a dominant negative regulator of the cGMP/NO cascade, thus blunting vasodilation and possible downstream hypertension.

From the above it appears that the effects on BP due to EPO exposure may be mediated by the generation of endothelial NO. At least there is some evidence for a reduction in NO generation and blunting of the NO effect on both the kidneys and vasculature. Transgenic models demonstrate that endothelial NO appears to be critical in maintaining normotension, preventing cardiovascular dysfunction and survival *in vivo* in the setting of EPO use.

### Catecholamines

In rabbit, aortic rings incubated with very high EPO concentrations result in increased vasoconstriction when exposed to norepinephrine [37, 53]. Norepinephrine concentrations were increased following 12 weeks of EPO treatment in one study [69] but decreased in another study of Japanese hemodialysis patients [70]. Vasoconstriction induced by infusion of norepinephrine was increased in patients with CKD after treatment with EPO, thus the vascular sensitivity to norepinephrine was increased in human studies [51, 71]. Furthermore, white blood cell α-2 receptor density was reduced following EPO treatment [45, 72]. In summary, these data suggest both an elevation of catecholamines as well as enhanced sensitivity to catecholamines on the blood vessels as a mechanism of EPO-induced hypertension.

### Renin–angiotensin–aldosterone system (RAAS)

Eggena *et al.* [73] evaluated the effects on the RAAS at the molecular level in Wistar rats upon treatment with EPO. In the kidney, both renin mRNA and angiotensinogen mRNA were increased by EPO. In the heart, no alterations in mRNAs were seen. In the aorta, angiotensinogen-mRNA but not renin mRNA was elevated. In both the aorta and kidney, a significant correlation was observed between angiotensinogen mRNA and BP. In cultured rat vascular smooth muscle cells, Barrett et al. [74] show that EPO induces an increase in both types of angiotensin II receptors, even in the presence of enalapril or losartan. Among hemodialysis patients, infusion of angiotensin II leads to excess vasoconstriction, suggesting that angiotensin II sensitivity is increased with EPO treatment [51]. These data suggest that the vasoconstrictive potential is enhanced with EPO treatment.

In isolated perfused rodent kidneys, EPO treatment results in RAAS-stimulated sodium retention [75]. This is in sharp contrast to normal human volunteers where EPO treatment caused a reduction in plasma volume, plasma renin activity and aldosterone [76]. However, in rats with subtotal nephrectomy treated with EPO, in terms of treatment there is no unique effect on BP lowering of RAAS blockade. This is because the reduction in systolic BP was similar when rats were treated with traditional triple therapy (reserpine, hydralazine and hydrochlorothiazide) or with the RAAS blockers captopril or losartan [36].

Despite an increase in mean arterial pressure in 12 Japanese hemodialysis patients treated with EPO, plasma renin activity was found to be reduced [70]. Among dialysis patients treated with EPO, a close relationship is seen between exchangeable sodium, an increase in plasma aldosterone and an increase in BP [77]. As discussed above, attention to dry weight can abrogate EPO-induced hypertension among dialysis patients.

The above data—both in animals and humans—do not exclude the possibility of EPO causing hypertension by inducing volume excess or in the setting of volume excess. For example, the rodent subtotal nephrectomy models are associated with volume overload, which may be a prerequisite for developing hypertension. EPO, as noted above, can provoke sodium retention as well. However, the quality of the data do not allow deducing a cause-and-effect relationship.

### Blood viscosity

Blood viscosity increases in parallel with blood hematocrit and has been cited as a mechanism of EPO-induced hypertension. However, not all patients who have correction of anemia get hypertensive. Thus the change in blood viscosity by itself appears to be insufficient to account for EPO-induced hypertension.

### Vascular sensing of hypoxia

Breathing 60% oxygen for 10–12 min leads to an increase in forearm vascular resistance paralleled by a reduction in forearm blood flow, but not in all patients [78]. This phenomenon is only observed in those patients in whom BP increases with EPO treatment [78]. Thus the vascular response to hypoxia—and its reversal with correction of anemia—may be a fundamental mechanism of the genesis of hypertension induced by EPO.

## HYPOXIA-INDUCIBLE FACTORS: PROLYL HYDROXYLASE INHIBITORS

EPO gene expression is provoked by hypoxia-inducible transcription factors (HIFs) [79]. HIF-α subunits are oxygen labile and, under normal oxygen tension, oxidized by prolyl hydroxylases. Inhibitors of prolyl hydroxylases can stabilize HIFs, simulate hypoxia and promote erythropoiesis [79]. HIF stablizers not only stimulate EPO, but also many other genes responsible for angiogenesis, tumor growth, cell proliferation and metabolism. HIF stablizers may aggravate hypertension by several mechanisms. For example, chronic intermittent hypoxia through HIF signaling in the carotid artery is thought to provoke systemic hypertension [79]. A 2016 study in *in vitro* and *in vivo* rodent models shows that hypoxia induces inorganic phosphorus–induced vascular smooth muscle calcification [80]. In this rodent model, roxadustat, an oral HIF prolyl hydroxylase inhibitor, enhanced vascular calcification [80]. The downstream effect of long-term use may therefore be hypertension. Vascular calcification is common in CKD and contributes to arterial stiffness. Increased arterial stiffness is strongly associated with elevated interdialytic ambulatory blood pressure [81]. On the other hand, HIF stablizers may raise EPO to more physiologic levels, reduce cross talk with vascular EPO receptors and mitigate hypertension. A 2014 study demonstrated that compared with EPO, treatment with the HIF stabilizer molidustat corrected anemia associated with subtotal nephrectomy, but in contrast to EPO, it reduced systolic BP in a dose-dependent manner [82]. The authors postulate that anti-inflammatory and antifibrotic effects of the drug on the kidney may be operative. This study further illustrates that increases in hematocrit can be dissociated from an increases in blood pressure.

Several HIF stablizers are in Phase 3 clinical trials for the treatment of anemia in CKD. The ongoing Phase 3 trials of HIF stabilizers are evaluating, among patients with CKD, the equivalence of hemoglobin increase from baseline compared with

approved EPO-stimulating agents. Whereas hypertension is not a primary outcome measure for these Phase 3 trials, the equivalence of the cardiovascular outcomes is an important safety endpoint.

Human data are available but are inadequate to address the question of hypertension with HIF stablizers. Among 145 patients with CKD not on dialysis who were treated with roxadustat for 16–24 weeks in varying doses, hypertension was reported in 11 (7.6%) [83]. Among 60 incident dialysis patients treated with roxadustat for 12 weeks, hypertension requiring an escalation of antihypertensive therapy occurred in 6 (10%) [84]. In the above studies, there was no placebo group or a group treated with EPO alone, therefore, whether the drug is equivalent to, safer or more detrimental than EPO or placebo with respect to hypertension remains unclear. In a 2017 study in China, among 61 CKD patients treated with roxadustat over 8 weeks, 4 (7%) developed hypertension compared with none of 30 treated with placebo [85]. In the same report, among 74 patients on dialysis treated for 6 weeks, 3 (4%) developed hypertension compared with 1 of 22 (5%) treated with EPO [85]. In a 2016 study of vadadustat among nondialysis CKD patients treated for 20 weeks, 138 were assigned to vadadustat and 72 to placebo; 11 in the vadadustat group (8%) and 2 in the placebo group (2.8%) had hypertension reported as an adverse event.

## CONCLUSIONS

In summary, mediated by a variety of molecules, there is an imbalance in the vascular tone favoring net vasoconstriction that mediates hypertension due to EPO, especially in the setting of CKD. Besides the direct effects of the prostanoids, endothelins and NO pathways, EPO administration is also associated with increased responsiveness to catecholamines and angiotensin II and mitigation of hypoxia-induced vasodilatation responses. EPO-induced hypertension, at least in part, appears to be independent of an increase in hemoglobin, because experiments show that hemoglobin may be increased by EPO without an increase in BP by simply treating the animals with EPO binding protein and that treatment with EPO in the setting of iron deficiency may not increase hemoglobin but may still increase BP. However, experimental data are not consistent across studies and better mechanistic designs are needed, especially in people with CKD, to dissect the precise mechanism of EPO-induced hypertension. Animal studies suggest that HIF stablizers may provoke hypertension and those with high phosphorus concentrations and sleep apnea may be at an increased risk. Others show that there may be an antihypertensive effect via kidney repair. Whether this class of drugs will reduce the risk of hypertension compared with EPO remains to be seen.

## CONFLICT OF INTEREST STATEMENT

R.A. serves on steering committees of clinical trials funded by Akebia, Johnson & Johnson, Relypsa, Bayer and Boehringer Ingelheim.

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